

Application No.: 10/500,240
Amendment Date: 19-Dec-07
Reply to Office Action of: 19-Jun-07

REMARKS/ARGUMENTS

Reconsideration of this application, as amended, is respectfully requested.

I. Status of the Claims and Specification

A. Status of the Claims

After entry of these amendments, Claims 1-3, 6-12, 14, 15, 17 and 59 are pending. Claims 4, 16, 18-58 and 60-65 are canceled. Claims 5 and 13 were canceled in the amendment dated March 23, 2007.

Claims 1 and 17 are amended to recite unicellular or filamentous fungi, rather than lower eukaryotic host cell. Support for this amendment is found in the application as filed, at page 11, lines 10-12.

Claim 1 is also amended to recite the step of "introducing into the host cell one or more nucleic acids encoding an α -1,2 mannosidase activity and a GnTI activity." Support for this amendment is found in the application as filed, at paragraph 0123, at original claim 7, and at Example 2.

Claim 1 is also amended to limit the variable "X" (in the formula GlcNAcMan_XGlcNAc₂) to 3 or 4.

Claims 18-45, 47-58 and 60-65, which were withdrawn from examination as covering a non-elected invention, are now canceled.

Claim 4 is canceled in response to an indefiniteness rejection.

Product-by-process claim 46 is canceled.

Claims 5 and 16 are canceled in view of the amendments to Claim 1.

Claim 6 is amended to delete subject matter withdrawn from examination (glycosyltransferase activity).

No new matter is added by these amendments.

B. Status of the Specification

The specification is amended to better describe Figures 4-15, 25, 26, 28, 29, and 32-34, and to refer to the Sequence identifiers as requested by the Examiner.

II. Objections to Information Disclosure Statement

The Examiner objects to the Information Disclosure Statement filed November 8, 2005, on the grounds that the reference CM2 (Segawa et al, 1999) was not submitted. In response, an

Application No.: 10/500,240
Amendment Date: 19-Dec-07
Reply to Office Action of: 19-Jun-07

Information Disclosure Statement, which includes the cited Segawa reference, is submitted with this amendment.

III. Objections to Specification

The Examiner objects to the specification on the grounds that the specification lacks reference to SEQ ID Nos. assigned to the sequences listed in the figures and throughout the specification (for example, at Figures 4 and 5 and at page 53, paragraph 173. In response, the specification is amended to include the sequence listing identifiers. In view of the action taken, it is believed that the objections to the specification have been overcome. It is respectfully requested that the objections be withdrawn.

IV. Notice to Comply with Sequence Listing Requirements

The Office Action also included a Notice to Comply with the Requirements For Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures, which requested an initial or substitute CRF and paper copy along with a statement that the content of paper copy and CRF are the same. Examiner acknowledges that a CRF, paper copy, and statement had been submitted 25 June 2004. All of the sequences in the application are contained with the sequence listing that had been submitted 25 June 2004. Therefore, it is believed that a substitute sequence listing (CFR, paper copy, and statement) and an amendment directing entry of the paper copy into the specification is unnecessary.

Nevertheless, applicants submit herewith a substitute CRF and paper copy, along with a statement that the content of paper copy and CRF are the same.

In view of the action taken, it is believed that the sequence listing requirements have now been satisfied.

V. Claim Objections

Claims 6 and 46 are objected to for including non-elected subject matter. The Examiner objects to the recitation of "glycosyltransferase" in claim 6, and the reference to withdrawn claim 44 in claim 46.

Claim 6 is amended to delete "glycosyltransferase."

Claim 46 is canceled.

In view of the action taken, it is believed that the claim objections have been overcome. It is respectfully requested that the objections be withdrawn.

VI. Obviousness-Type Double Patenting Rejections

A. Rejection over claims 1, 3 and 8-12 of U.S. Pat. No. 7,029,872

Claims 1-4, 6, 7, 10, 11 and 14-17 stand rejected under the judicially created doctrine of obviousness-type double patenting over claims 1, 3 and 8-12 of U.S. Pat. No. 7,029,872.

According to the rejection, the claims of the '872 patent "are a species of the instant claims."

Applicants respectfully traverse the double patenting rejection, on the grounds that there is a patentable distinction between the claims of this application and the claims of the '872 patent. While both the '872 and the instant application claim methods for making hybrid and complex N-glycans in lower eukaryotes, the claimed method in the '872 patent is patentably distinct from the method claimed in the instant application.

Independent claims 1 and 3 of the '872 patent relate to a method for making hybrid N-glycans in a host cell wherein the host cell does not display alpha-1,6-mannosyltransferase activity with respect to an N-glycan on a glycoprotein. In other words, the claims require that the host cell does not transfer mannosidase to the mannose on the 1,3 arm of the N-glycan attached to the asparagine of a glycoprotein. The mannose is attached in an alpha-1,6 linkage; hence the name alpha-1,6-mannosyltransferase.

In the instant application, the claims are drawn to a method for making complex N-glycans in which the host cell is engineered to have diminished or depleted activity of one or more enzymes that transfers a mannose to the mannose on the 1,6 arm of the glycan while it is still attached to a membrane lipid and not to the glycoprotein. These enzymes are known as dolichyl-P-Man:Man5GlcNAc2-PP-dolichyl alpha-1,3 mannosyltransferase (Alg3); dolichyl-P-Man:Man6GlcNAc2-PP-dolichyl alpha-1,2 mannosyltransferase (Alg9) and dolichyl-P-Man:Man7GlcNAc2-PP-dolichyl alpha-1,6 mannosyltransferase (Alg12). These enzymes are all involved in building a Man9GlcNAc2 lipid-linked glycan that is attached to a membrane lipid by adding mannoses to other mannoses on the 1,6 arm of the lipid-linked glycan. In normal cells, after the lipid-linked Man9GlcNAc2 is made, it is transferred from the lipid membrane to the asparagine of a protein. After the glycan is on the protein, the Man9GlcNAc2 structure is trimmed by an endogenous mannosidase that removes just one mannose from the Man9GlcNAc2 N-glycan to make a Man8GlcNAc2 N-glycan. It is to this Man8GlcNAc2 N-glycan that the alpha-1,6-mannosyltransferase can add a mannose in an alpha-1,6 linkage to the mannose on the 1,3 arm of the N-glycan. However, in the instant application, diminishing or depleting the activity of the Alg3 gene, only a lipid-linked N-glycan with an aberrant Man5GlcNAc2 structure is made. It is this structure that is transferred to the asparagine of a protein.

Application No.: 10/500,240
Amendment Date: 19-Dec-07
Reply to Office Action of: 19-Jun-07

In the '872 patent, in Claim 1, a Man₅GlcNAc₂ structure that is attached to an asparagine of a protein (as described above) is treated with an alpha-1,2-mannosidase that removes mannoses in attached alpha-1,2 linkages to produce N-glycans that have a Man₅GlcNAc₂ structure. Treating the structure with GnT I as in Claim 3 results in an N-glycan with a GlcNAc₂Man₅GlcNAc₂ structure. In contrast, in the currently claimed method, the lipid-linked glycan has an aberrant Man₅GlcNAc₂ structure, which is transferred to the asparagine of a protein. Treating this aberrant Man₅GlcNAc₂ structure with an alpha-1,2-mannosidase produces N-glycans that have a Man₃GlcNAc₂ structure. Treating the Man₃GlcNAc₂ structure with GnT I produces N-glycans that have a GlcNAcMan₃GlcNAc₂ structure.

Thus, the claimed in the instant application claims a method that is distinct from the method claimed in the '872 patent. Accordingly, it is requested that the double patenting rejection over the '872 patent be withdrawn.

B. Rejection over claims 1, 3, 8, 12, 13, 16-18 and 28 of copending Appl. Ser. No. 10/371,877

Claims 1-4, 8-11, 14-17, and 59 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting over claims 1, 3, 8, 12, 13, 16-18, and 28 of copending patent application U.S. serial no. 10/371,877. A copy of the pending claims of application serial no. 10/371,877, as set forth in the October 26, 2007 amendment, is attached at Exhibit A.

The rejection states that both sets of claims are drawn to making N-glycan recombinant glycoprotein in a eukaryotic host cell. The rejection states that the '877 application claims are narrower than the pending claims, because the '877 claims recite "the alpha-1,2 mannosidase catalytic domain." The Examiner also notes the recitation of "NeuNAc-Gal-GlcNAc-Man ('877, claim 13; '240 claim 15), and the use of *P. pastoris* ('877 claim 16; '240 claim 17).

Applicants respectfully traverse the double patenting rejection, on the grounds that there is a patentable distinction between the claims of this application and the '877 application claims for the same reasons as discussed with respect to the '872 patent.

Further, the cited claims of the '877 application require production of a Man₅GlcNAc₂ N-glycan structure. In contrast, the claims of this application as now presented recite production of N-glycan having an GlcNAcMan_XGlcNAc₂ core structure, wherein X is 3 or 4.

Accordingly, it is requested that the double patenting rejection over the '877 application be withdrawn.

Application No.: 10/500,240
Amendment Date: 19-Dec-07
Reply to Office Action of: 19-Jun-07

C. Rejection over claims 1-15 and 18-20 of copending Appl. Ser. No. 11/187,066

Glycoprotein claim 46 is provisionally rejected under the judicially created doctrine of obviousness-type double patenting over claims 1-15 and 18-20 of copending patent application Serial No. 11/187,066.

Claim 46 is canceled, thereby obviating the double patenting rejection over the '066 application. Hence, it is requested that the double patenting rejection over the '066 application be withdrawn.

VII. Rejections Under 35 U.S.C § 112, First Paragraph

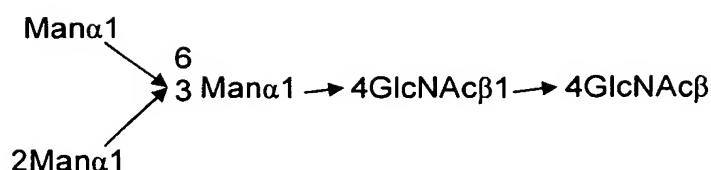
Claims 1-4, 6-12, 14-17, 46 and 59 were rejected under 35 U.S.C. § 112, first paragraph, under the enablement requirement of the first paragraph. In essence, the rejection appears to state that the application does not reasonably enable "a method of producing a recombinant glycoprotein having N-glycans comprising GlcNAcMan_xGlcNAc₂ core structure, wherein X is 3, 4, 5 wherein the method comprises disrupting one or more enzymes that transfers a sugar residue to the 1,6 arm of a lipid-linked oligosaccharide structure in any host cell and expressing any glycosidase activity."

Claim 1 has been amended to recite

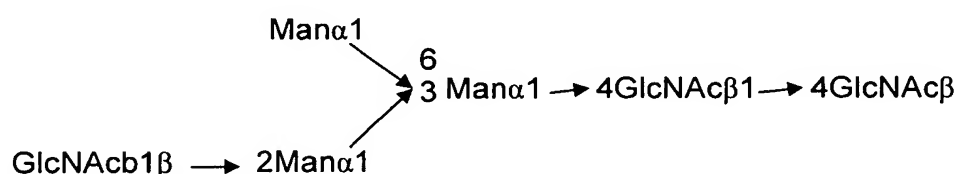
A method for producing a recombinant glycoprotein in a non-human eukaryotic unicellular or filamentous fungus host cell that expresses a glycosidase activity, the method comprising the step of diminishing or depleting the activity of one or more enzymes in the host cell that transfers a sugar residue to the 1,6 arm of a lipid-linked oligosaccharide structure, and introducing into the host cell one or more nucleic acids encoding an α -1,2-mannosidase and a GnT I activity; wherein said method results in the production within the host cell of recombinant glycoproteins having N-glycans attached thereto comprising GlcNAcMan_xGlcNAc₂ core structures, wherein X is 3 or 4.

It is believed that currently amended Claim 1 (and its dependent claims) are reasonably enabled by the specification. The applicants teach how to modify the glycosylation pathway in a host cell to enable a host that does not normally produce complex, human-like N-glycans to be able to produce complex, human-like N-glycans.

In nearly all eukaryotes, glycoproteins are derived from the common core oligosaccharide precursor Glc₃Man₉GlcNAc₂-PP-Dol, where PP-Dol stands for dolichol-pyrophosphate (Figure 1). Within the endoplasmic reticulum, synthesis and processing of dolichol pyrophosphate bound oligosaccharides are identical between all known eukaryotes. However, further



The applicants further teach that introducing a GnT I into the cell results in the production of the following GlcNAcMan₃GlcNAc₂ N-glycan.



The applicants further teach that adding other mammalian glycosylation enzymes to the host enable the production of other complex N-glycans such as GlcNAc₂Man₃GlcNAc₂, Gal₂GlcNAc₂Man₃GlcNAc₂, and NANA₂Gal₂GlcNAc₂Man₃GlcNAc₂.

The applicants provide working examples where they teach how to genetically engineer a host cell such as *Pichia pastoris* to enable the host cell to produce glycoproteins that have at least a GlcNAcMan₃GlcNAc₂ N-glycan (See, Figure 18). The applicants further teach a library approach that has facilitated identifying suitable catalytic domain/targeting peptide combinations that enable construction of host cells that can make the above hybrid or complex N-glycans. Using the library approach and *Pichia pastoris* as a model, the applicants show how to construct host cells that made GlcNAcMan₃GlcNAc₂ N-glycan.

It is believed that a person skilled in the art, having read the instant application, would comprehend and be able to make and/or otherwise identify a variety of unicellular or filamentous fungi host cells lacking enzyme activity that transfers a sugar residue to the 1,6 arm of a lipid-linked oligosaccharide structure using techniques well-known in the art and then using the methods taught in the instant application to genetically engineer any lower eukaryote host cell to be capable of making glycoproteins having at least a GlcNAcMan₃GlcNAc₂ N-glycan structure.

Thus, a person skilled in the art in view of the specification would understand that the applicant's invention comprises any lower eukaryote host cell that is deficient in enzyme activity that transfers a sugar residue to the 1,6 arm of a lipid-linked oligosaccharide structure and includes any alpha-1,2 mannosidase and any GnT I activity that is capable of making

Application No.: 10/500,240
Amendment Date: 19-Dec-07
Reply to Office Action of: 19-Jun-07

glycoproteins wherein the N-glycan species is at least GlcNAcMan₃GlcNAc₂. One skilled in the art would understand that the importance of the applicant's invention is that for the first time, host cells are provided that can be used to make glycoproteins wherein the majority (if not all) of glycoproteins have a particular human-like N-glycan structure.

In conclusion, the application as filed sufficiently teaches a person skilled in the art how to engineer or select lower eukaryotic cells that do not display enzyme activity that transfers a sugar residue to the 1,6 arm of a lipid-linked oligosaccharide structure and which further include nucleic acids encoding an α -1,2 mannosyltransferase and GnT I to alert the person skilled in the art that the applicant was in possession of the invention as of the filing date of the application.

At page 10, the Examiner objects to the recitation of "any host cell and expressing any glycosidase activity." Claim 1 is now amended to recite the host cell of a "unicellular or filamentous fungus," and the glycosidase activities of "an α -1,2 mannosidase activity and a GnTI activity."

At page 13, the Examiner challenges enablement of claims reciting the use of GnTII. However, as noted above, the claims as now presented require introducing into the host cell one or more nucleic acids encoding a GnT I activity, rather than GnTII.

At pages 13-14, the Examiner challenges enablement of glycoproteins having a sialic acid (NeuNAc) component, (such as claims 14 and 15). The Examiner cites Wildt and Gerngross, *Nature Reviews: Microbiology* 3:119-128 (2005), and states that "the enzyme used to sialate sugars would need to be targeted to appropriate site in the Golgi and the substrates for the reaction would need to be at the appropriate site in the Golgi." Presumably, the Examiner is referring to the statements at page 127 of Wildt et al., entitled "Sialic acid transfer – the final step." Wildt et al. states that:

Three criteria have to be met to allow for the production of sialylated glycoproteins in yeast: the availability of a terminal-galactose containing N-glycan . . . , an active sialyltransferase and a pool of CMP-sialic acid.

Wildt et al states that the results "are preliminary and need to be further improved before quantitative in vivo sialylation can be demonstrated." The statement does not suggest that one skilled in the art would not be able to practice the steps of sialylation taught in the specification.

Thus, the scope of the currently amended claims are believed to supported by the specification. Reconsideration of the rejection is requested.

Application No.: 10/500,240
Amendment Date: 19-Dec-07
Reply to Office Action of: 19-Jun-07

VIII. Rejections Under 35 U.S.C § 112, Second Paragraph

Claim 4 stands rejected under 35 U.S.C § 112, second paragraph, as indefinite. The Examiner states that the recitation of producing an N-glycan does not introduce any new limitations into independent claim 1. In response, claim 4 is canceled, thereby obviating the indefiniteness rejection.

IX. Rejections Under 35 U.S.C. § 102

A. Rejection of Method Claims

The currently claimed method of the invention is fundamentally different from the processes described in both the Gerngross '872 patent and in Chiba. Claim 1 of the instant application has been amended to recite

A method for producing a recombinant glycoprotein in a unicellular or filamentous fungus host cell that expresses a glycosidase activity, the method comprising the step of diminishing or depleting the activity of one or more enzymes in the host cell that transfers a sugar residue to the 1,6 arm of a lipid-linked oligosaccharide structure, and introducing into the host cell one or more nucleic acids encoding an α -1,2 mannosidase activity and a GnTI activity; wherein said method results in the production within the host cell of recombinant glycoproteins having N-glycans attached thereto comprising GlcNAcMan_XGlcNAc₂ core structures, wherein X is 3 or 4.

The currently amended claims provide a method for producing a recombinant glycoprotein in a unicellular or filamentous fungus host cell. The currently amended claims require the step of "diminishing or depleting the activity of one or more enzymes in the host cell that transfers a sugar residue to the 1,6 arm of a lipid-linked oligosaccharide structure" and further require "introducing into the host cell one or more nucleic acids encoding an α -1,2 mannosidase activity and a GnTI activity, " thereby producing in the host cell "recombinant glycoproteins having N-glycans attached thereto comprising GlcNAcMan_XGlcNAc₂ core structures, wherein X is 3 or 4."

As explained below, neither of the cited references, Gerngross '872 or Chiba, describe the claim limitation of "diminishing or depleting the activity of one or more enzymes in the host cell that transfers a sugar residue to the 1,6 arm of a lipid-linked oligosaccharide structure." Instead,

both Gerngross '872 and Chiba add a sugar residue to the 1,6 arm of a protein-bound oligosaccharide.

1. Rejection over U.S. Pat. No. 7, 029,872, to Gerngross

Claims 1-4, 6, 7, 10, 11, 14-17 and 46 stand rejected under 35 U.S.C. § 102(e) as anticipated by U.S. Pat. No. 7, 029,872, to Gerngross.

The rejection states that Gerngross "describes a method of making recombinant glycoprotein comprising an N-glycan structure in a eukaryotic host cell, wherein the method comprises diminishing or depleting one or more enzymes that transfer a sugar residue to the mannose forming the 1,6 arm of a lipid-linked oligosaccharide structure." However, the Gerngross method uses a host cell that does not display an enzyme activity that transfers a mannose to the mannose forming the 1,3 arm of a protein-linked oligosaccharide structure.

In contrast, the instant application comprises diminishing or depleting one or more enzymes that transfers a sugar residue to the mannose forming the 1,6 arm of a lipid-linked oligosaccharide structure. This is a fundamental and clearly distinguishable difference between the method of Gerngross from the currently claimed method.

Gerngross describes glycosylation of oligosaccharides by mannosyltransferases residing in the Golgi. See col 10, l. 35-38. Gerngross states that "mutants of *S. cerevisiae*, deficient in mannosyl transferase activity (e.g. och1 or mnn9 mutants) have shown to be non-lethal and display a reduced mannose content in the oligosaccharide of yeast glycoproteins." Col. 10, l. 41-44.

Gerngross is predominantly directed to producing glycoproteins having a $\text{Man}_5\text{GlcNAc}_2$ core structure (see, e.g., col. 11-12). The only references to a $\text{GlcNAc}_3\text{Man}_2$ structure are in Example 3, which describes engineering of a strain with a mannosidase II, and in Table 6, at column 24. The third to sixth entries in Table 6 describe oligosaccharide structures having $\text{Man}_{3,4}\text{GlcNAc}_2$ cores structures. Each describes gene deletions at OCH1, MNN4 and MNN6. Gerngross describes formation of a $\text{GlcNAcMan}_3\text{GlcNAc}_2$ structure at Figure 1.B after action of the enzyme mannosidase II, in the Golgi, when the oligosaccharide is bound to the protein. Table 6, at column 24, also describes a method of forming a $\text{GlcNAc}_{2-4}\text{Man}_3\text{GlcNAc}_2$ core structures. Here, again, the formation of this oligosaccharide requires action of the enzyme mannosidase II, when the oligosaccharide is bound to the protein. Further, while Claim 4 of Gerngross results in production of a $\text{GlcNAcMan}_3\text{GlcNAc}_2$ N-glycan, the method in claim 4 of Gerngross requires the action of a mannosidase II to produce the

Application No.: 10/500,240
Amendment Date: 19-Dec-07
Reply to Office Action of: 19-Jun-07

GlcNAcMan₃GlcNAc₂ N-glycan. Thus, the method of Gerngross and the currently claimed method of the instant application are clearly distinguishable.¹

In light of the above, it is believed that currently amended Claims 1-4, 6, 7, 10, 11, 14-17 are not anticipated by Gerngross. Reconsideration of the rejection is requested.

2. Rejection over Chiba et al, *J Biol. Chem* 1998, 273:26298-26304

Claims 1-4, 10-12, 15-17 and 46 stand rejected under 35 U.S.C. § 102(b) as anticipated by U.S. Pat. No. 7, 029,872, to Chiba (*J Biol. Chem* 1998, 273:26298-26304). The rejection states that Chiba et al. "teach that CPY was used as a reporter glycoprotein to assess the glycosylation pattern of a triple mutant strain of yeast, wherein the yeast was a mutant for Och1, Mnn1, and Mnn4." The Examiner contends that "Chiba et al. meet all the steps" of claim 1.

Applicants traverse, on the grounds that Chiba does not meet a key limitation of currently amended claim 1, the step of "diminishing or depleting the activity of one or more enzymes in the host cell that transfers a sugar residue to the 1,6 arm of a lipid-linked oligosaccharide structure."

Chiba discloses the production of glycoproteins that Man₅GlcNAc₂ N-glycans in yeast in which Och1, mnn1, and Mnn4 genes had been disrupted, and in an alpha-1,2-mannosidase has been introduced to trim the Man₈GlcNAc₂ structure to the Man₅GlcNAc₂ structure. Hence, Chiba does not teach the claimed method.

In light of the above, it is believed that currently amended claims 1, 4, 10-12, and 15-17 are not anticipated by Chiba. Reconsideration of the rejection is requested.

B. Rejections of Product-by-Process Claim 46

Product-by-process claim 46 stands rejected over each of Kornfeld, *J Biol. Chem* 1983, 258:7907-7910, Wagner et al, *Glycobiology* 1996 6:165-175, and Velasco et al, *J. Cell Biol* 1993 122:39-51.

Claim 46 is canceled, thereby obviating these rejections.

In view of the action taken and arguments made, it is believed that the anticipation rejections have been overcome. It is respectfully requested that the rejections be withdrawn.

XI. Conclusion

¹ The rejection states at page 16 of the office action that Gerngross "has a common inventor with the instant application." However, that is incorrect. Gerngross is the sole inventor of the '872 patent but is not an inventor of the claimed invention.

Application No.: 10/500,240
Amendment Date: 19-Dec-07
Reply to Office Action of: 19-Jun-07

In view of the action taken and arguments made, pending claims 1-3, 6-12, 14, 15, 17 and 59 are enabled by the specification, and are not anticipated by the cited prior art. Further, pending claims 1-3, 6-12, 14, 15, 17 and 59 are not subject to obviousness-type double patenting. Pending claims 1-3, 6-12, 14, 15, 17 and 59 are now in condition for allowance.

Favorable action is earnestly solicited.

Respectfully submitted,

By

John C. Todaro, Reg. No. 36,036
Attorney for Applicants

MERCK & CO., Inc.
P.O. Box 2000
Rahway, New Jersey 07065
Tel.: (732) 594-0125

Date: December 19, 2007